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Potassium channel blockers differentially affect carbachol and (—)-N^6-phenylisopropyladenosine on guinea-pig atria

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1 The effect of three different potassium channel blockers (tetraethylammonium, TEA; 4-aminopyridine, 4-AP; and apamin) and of variations in the concentration of K^+ and Ca^{2+} in the medium, have been studied on the responses of guinea-pig isolated atria to (−)-N^6-phenylisopropyladenosine (R-PIA), a stable adenosine A_1-receptor agonist, and to carbachol, a muscarinic agonist. R-PIA and carbachol showed the same negative inotropic effects over a similar range of concentrations (3–300 μM), both in spontaneously beating and in electrically driven atria.

2 TEA (0.1 to 20 mM) and 4-AP (0.3 to 3 mM), both antagonized the negative inotropic and chronotropic effects of carbachol in a concentration-dependent manner. In contrast, these compounds failed to inhibit the effects induced by R-PIA. Apamin, a specific blocker of a low conductance Ca^{2+}-activated K^+ channel, was ineffective in accordance with the absence of these channels in atrial tissue.

3 TEA (0.1 to 20 mM) inhibited the negative inotropic effect of carbachol, but not that of R-PIA, in atria paced and depolarized by a high K^+ medium (22 mM). In this preparation Na^+ current is abolished and the contraction induced by noradrenaline and electrical stimulation is solely dependent on Ca^{2+} influx currents.

4 Stepwise addition of Ca^{2+} to a calcium-depleted perfusing medium of electrically driven atria, induced a positive inotropic effect which was inhibited by R-PIA. In contrast, carbachol had no effect.

5 In agreement with our previous study, the data suggest that R-PIA acts on isolated atria by inhibiting Ca^{2+} influx through L-channels.

Introduction

Similar effects of adenosine and acetylcholine were observed in the early studies of atrial preparations (Johnson & McKinnon, 1956; Hartzell, 1979). In atrial cells and in the isolated SA node, adenosine (Ado) and acetylcholine (ACh) shorten the action potential, produce hyperpolarization and depression of automaticity (Belardinelli & Isenberg, 1983; West & Belardinelli, 1985). The similar effect of the two drugs is dependent on activation of K^+ channels that in turn indirectly reduce the inward calcium flux during the action potential (reviews: Nawrath *et al.*, 1985; Isenberg *et al.*, 1987; Sperelakis, 1987; West *et al.*, 1987). Although Ado and ACh act on different receptors, these may be connected with the same population of K^+ channels, via guanosine 5'-triphosphate (GTP)-binding proteins (Kurachi *et al.*, 1986; Böhm *et al.*, 1986; Cerbai *et al.*, 1988). In previous studies (Caparrotta *et al.*, 1987; Borea *et al.*, 1989) we observed that in isolated atria, stable analogues of adenosine antagonized the positive inotropic effect of Bay K 8644, a dihydropyridine Ca^{2+} L-channel activator. Carbachol, a stable cholinoceptor agonist, was ineffective. The most simple explanation was that Ado analogues and carbachol, though having similar effects in single cells, may act differently in integrated structures such as isolated atria. In view of this, the aim of the present work was to compare the effect of different potassium channel blockers and of ionic variations in the medium, on the negative inotropic actions of

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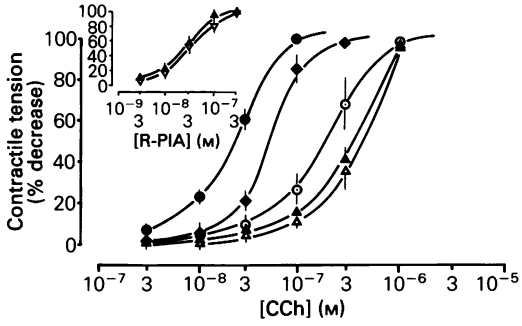

Figure 1 Inhibitory effect of tetraethylammonium (TEA) on the negative inotropic effect of carbachol (CCh) in spontaneously beating atria. Cumulative concentration-response curves for CCh in the absence (●) and in the presence of TEA 0.1 mM (◆), 1.0 mM (○), 10 mM (▲), 20 mM (△). Each point is the mean of 6–8 experiments. Vertical lines indicate s.e.mean. Inset: cumulative concentration-response curves for (−)-N^6-phenylisopropyladenosine (R-PIA) alone (▲) and in the presence of TEA 20 mM (▽).

(−)-N^6-phenylisopropyladenosine (R-PIA), an adenosine A_1-agonist, and of carbachol, in guinea-pig isolated atria.

The results show a different behaviour of carbachol and R-PIA when ionic channels and fluxes are modified by K^+ channel blocking drugs or by a change in the ion concentration. In these conditions, R-PIA, but not carbachol, is apparently able to inhibit the Ca^{2+} influx through L-channels.

Methods

The hearts were removed from guinea-pigs of either sex (300–500 g) and placed in a physiological solution (29°C) of the following composition (mM): NaCl 120, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.09, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5, and gassed with 95% O_2 + 5% CO_2. The atria were dissected, suspended in a 30 ml organ bath and connected to a transducer (Basile, type DYO). An initial tension of 1 g was applied to the tissue and changes in isometric tension were recorded by a writing oscillograph (Basile, Unirecord System, cod. 7050).

Left atria were mounted on punctate electrodes with a load of 0.5 g and stimulated by square wave electrical pulses (1 Hz, 3 ms, 0.5–1.2 V) provided by a Grass stimulator (Mod. 25 S4KR). The voltage was about 20% greater than threshold. The control developed tension ranged from 0.8 to 1.3 mN. An equilibration period of 60 min was allowed before experiments were started. To investigate the calcium-dependent responses, the fast Na^+ channels were inactivated by elevating the external potassium concentration to 22 mM (Pappano, 1970; Thyrum, 1974; Sada *et al.*, 1986); equimolar NaCl was subtracted to maintain constant osmolarity. Under these conditions, contractility disappeared. After 60 min equilibration in high K^+ (22 mM) bathing solution, calcium-dependent action potentials, with consequent developed tension were induced by the addition of noradrenaline 3 μM. Stimulation frequency was lowered ten fold while voltage was increased.

Concentration-response curves were constructed by cumulative addition of drugs. Drug responses were allowed to equilibrate (4–8 min) before the subsequent addition of a higher concentration.

Results are expressed as % decrease from the control tension and rate.

Drugs and compounds used

(−)-N^6-phenylisopropyladenosine (R-PIA) (Boehringer, Mannheim) was dissolved and diluted in 50% ethanol-50% bathing solution. The total volume of ethanol never exceeded 0.05% (12.5 μl per 30 ml) in the organ bath. Carbachol, tetraethylammonium, noradrenaline (NA) and 4-aminopyridine (4-AP) were from Sigma; apamin from Serva Feinbiochemic; tetraethylammonium chloride (TEA) from Aldrich. Fresh stock solutions were prepared in distilled water and subsequently diluted with bathing solution to achieve the desired concentration.

Analysis of results

Values are presented as means ± s.e.mean. The −log concentration that produced half-maximal effects (−log EC_{50}) and its s.e.mean were determined by interpolation according to Tallarida & Murray (1987).

Results

Negative effect of (−)-N^6-phenylisopropyladenosine and carbachol on contractile tension and frequency in spontaneously beating atria

(−)-N^6-phenylisopropyladenosine, (R-PIA), a stable A_1-adenosine receptor agonist produced negative inotropic (EC_{50} = 19 nM) and chronotropic effects (EC_{50} = 42 nM) on the guinea-pig spontaneously beating atria. These effects were concentration-dependent between 3 and 300 nM. Carbachol, a muscarinic agonist also showed negative inotropic (EC_{50} = 22 nM) and chronotropic (EC_{50} = 36 nM) effects in the same range of concentrations as R-PIA.

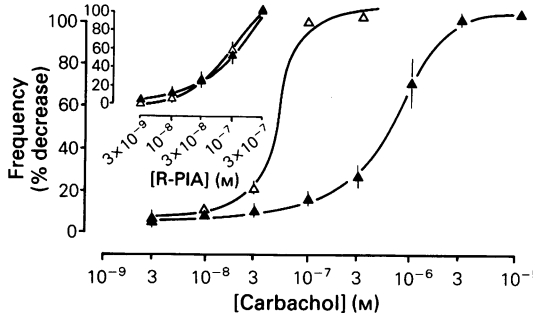

Figure 2 Effect of tetraethylammonium 20 mM (▲) on the negative chronotropic effect induced by carbachol (△, larger figure) and (−)-N^6-phenylisopropyladenosine (R-PIA, △, inset) in spontaneously beating atria. Each value is the mean of 8–12 experiments. Vertical lines indicate s.e.mean.

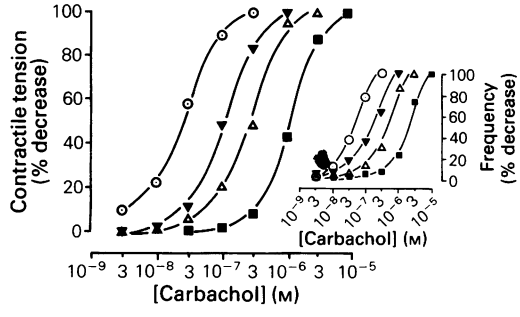

Figure 3 Concentration-response curves for carbachol alone (○) and in the presence of 4-aminopyridine 0.3 mM (▼), 0.7 mM (△) and 3 mM (■). Larger figure: % decrease of contractile tension. Inset: % decrease of frequency.

Tetraethylammonium and 4-aminopyridine on the negative inotropic and chronotropic effects of carbachol and R-PIA in spontaneously beating atria

Tetraethylammonium chloride 20 mM added to the medium 20 min before carbachol or R-PIA, slightly reduced *per se* the atrial rate (4 ± 1% of control) and increased the contractile tension (17 ± 2%), but maximally antagonized both the negative inotropic (Figure 1) and chronotropic (Figure 2) effects of carbachol. The concentration-response curve in the presence of TEA (0.1 mM to 10 mM) showed, in fact, that the inhibitory effect of carbachol was concentration-dependent (Figure 1). The slope of the Schild plot for contractility was different from unity (0.46 ± 0.13). The apparent pA_2 value was 4.47 ± 0.56 for contractility. TEA, 20 mM, failed to inhibit both the decrease of contractile tension (Figure 1) and of frequency (Figure 2) induced by PIA.

Concentrations of TEA higher than 20 mM could not be tested because of the development of arrhythmias, as also shown in other studies (Freeman, 1979; Asano *et al.*, 1985).

4-Aminopyridine (0.3 to 3 mM) was added to the organ bath 20 min before carbachol and R-PIA. 4-AP was found to cause *per se* some decrease in frequency and increase in contractility; this effect faded rapidly and the atria returned to the basal values in 2–6 min. 4-AP antagonized the negative inotropic and chronotropic effects of carbachol in a concentration-dependent manner and induced a parallel shift to the right of the dose-response curve of the drug (Figure 3). The slope of the Schild plot for contractility and frequency was not different from unity (1.10 ± 0.07 and 1.20 ± 0.15, respectively) possibly indicating a competitive antagonism. The

Table 1 Effects of 4-aminopyridine (4-AP), on the negative inotropism and chronotropism of (−)-N^6-phenylisopropyladenosine (R-PIA) in guinea-pig spontaneously beating atria

	*pD_2 ± s.e.mean (EC_{50})	
Compounds	*Contractile tension*	*Frequency*
R-PIA	7.70 ± 0.070 (19 nM)	7.37 ± 0.104 (42 nM)
R-PIA + 4-AP 0.3 mM	7.86 ± 0.134 [NS] (13 nM)	7.51 ± 0.096 [NS] (30 nM)
R-PIA + 4-AP 0.7 mM	7.87 ± 0.129 [NS] (19 nM)	7.54 ± 0.045 [NS] (28 nM)
R-PIA + 4-AP 3 mM	7.63 ± 0.075 [NS] (23 nM)	7.37 ± 0.095 [NS] (42 nM)

* pD_2 is the −log of the concentration able to induce half-maximum effect (EC_{50}). Each value was obtained by least squares method from 6–10 different curves.
NS = not significant vs R-PIA.

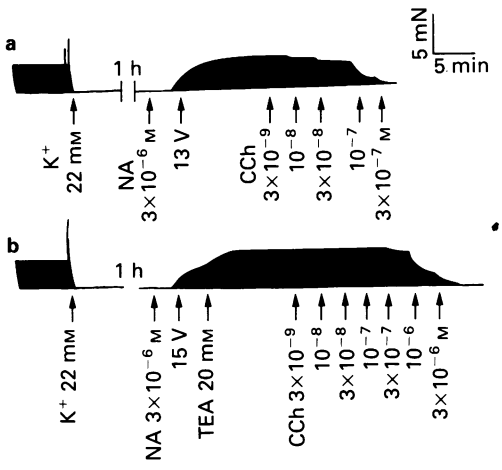

Figure 4 Effect of carbachol (CCh) in the absence (a) and presence (b) of tetraethylammonium (TEA) 20 mM on the contraction induced by noradrenaline (NA) 3 μM in the potassium-depolarized, electrically driven, left atria. Carbachol was added cumulatively and its final concentrations in the medium are indicated.

apparent pA_2 values were 4.02 ± 0.06 for contractility and 3.8 ± 0.10 for frequency, corresponding to K_is of 94 μM and 157 μM, respectively. 4-Aminopyridine 0.3 to 3 mM failed to inhibit both the inotropic and chrotropic effects of R-PIA (Table 1).

Apamin, 10–100 nM, preincubated 20 min before drug addition did not interfere with the negative ino-

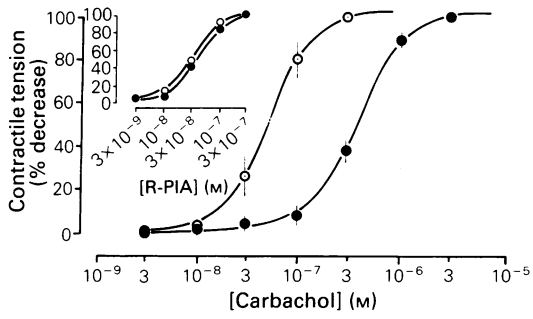

Figure 5 Effect of tetraethylammonium (TEA) on the negative inotropic effect of carbachol and of (−)-N^6-phenylisopropyladenosine (R-PIA, inset) in depolarized left atria (see Methods). Cumulative concentration-response curves for carbachol or R-PIA (inset) in the absence (○) and presence (●) of TEA 20 mM. Inotropic effects were expressed as percentage of the maximum increase induced by noradrenaline 3 μM. Each point is the mean of 6–7 experiments. Vertical lines indicate s.e.mean.

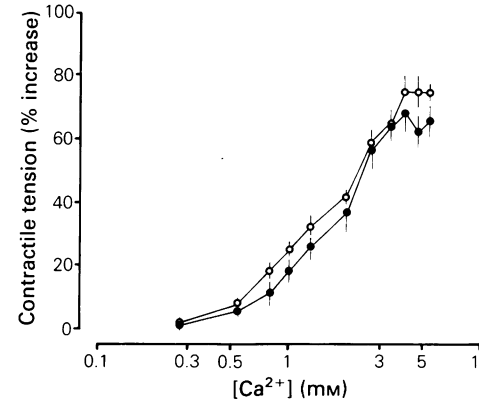

Figure 6 Effect of carbachol on the positive inotropic effect of Ca^{2+} in electrically driven left atria. Cumulative concentration-response curves for calcium in the absence (○) and presence (●) of carbachol 50 nM. Each point is the mean of 6 experiments. Vertical lines indicate s.e.mean.

tropic and chronotropic effects of carbachol and R-PIA (data not shown).

Tetraethylammonium chloride on the negative inotropic effect of carbachol and R-PIA in depolarized electrically driven atria

The effects of R-PIA and carbachol were investigated on guinea-pig left atria which were paced and depolarized (Figure 4). K^+ 22 mM abolishes the contractility of atria by inhibiting the fast Na^+ current (Thyrum, 1974). Further addition of catecholamines (or of other positive inotropic agents such as Ca^{2+}, or histamine) together with suitable conditions of electrical stimulation, induces slow action potentials chiefly due to Ca^{2+} currents (Pappano, 1970; Thyrum, 1974; Sada *et al.*, 1986). In our experiments, slow action potentials were evoked by adding noradrenaline 3 μM. In such conditions, R-PIA and carbachol showed the same effects as in non-depolarized atria; both drugs inhibited contractile tension (Figure 5) in a range of concentrations similar to those effective in spontaneously beating atria. TEA 20 mM induced *per se* an increase of contractile tension, probably due to an increase of Ca^{2+} influx (Asano *et al.*, 1985). TEA 20 mM inhibited the negative inotropic effect of carbachol (Figure 5) but not that of R-PIA (Figure 5), as previously shown in spontaneously beating atria.

Influence of calcium on the effects of R-PIA and of carbachol in electrically driven atria

In electrically driven atria the effect of R-PIA and carbachol on the contraction induced by progressive

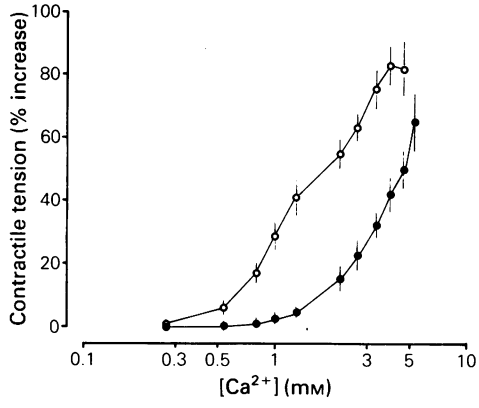

Figure 7 Inhibitory effect of (−)-N^6-phenylisopropyladenosine (R-PIA) on the positive inotropic effect of Ca^{2+} in electrically driven left atria. Cumulative concentration-response curves for calcium in the absence (○) and in presence (●) of R-PIA 50 nM. Each point is the mean of 6 experiments. Vertical lines indicate s.e.mean.

addition of Ca^{2+}, to a physiological medium previously depleted of this ion, was studied. In the absence of calcium the atrial contractility was abolished. Stepwise cumulative addition of Ca^{2+}, up to 4 mM, induced a concentration-dependent positive inotropic effect (Figure 6). Preincubation with carbachol 50 nM (EC_{75}) did not significantly affect the Ca^{2+}-dependent increase of contractile tension (Figure 6). In contrast, preincubation with R-PIA 50 nM (EC_{75}) clearly inhibited the Ca^{2+}-induced positive effect on contractile tension (Figure 7), mainly at lower Ca^{2+} concentrations (0.5–2.0 mM).

Discussion

Several types of potassium channel occur within the same cell (reviews: Hille, 1984; Cook, 1988). In cardiac tissue, at least seven potassium channels are operative (Pelzer & Trautwein, 1987). These have the function of repolarizing or hyperpolarizing the membrane potential at different moments and in the various cells. TEA blocks the majority of K^+ channels (see: Hille, 1984; Cook, 1988) such as the fast transient K^+ channels, delayed outward rectifier channels, and inward rectifiers. 4-AP is also non-selective, even if more active on transient delayed rectifier channels. In contrast, apamin, a bee venom polypeptide, is a selective blocker of the low conductance Ca^{2+}-activated K^+ channels which are insensitive to TEA and to 4-AP (Romey & Lazdunski, 1984; Cook & Haylett, 1985; Blatz & Magleby, 1986; Lazdunski *et al.*, 1987). Apamin was used in our experiments, as a negative control, because the potassium channels sensitive to this agent are present only in Purkinje fibres (Pelzer & Trautwein, 1987). The lack of effect by apamin on responses to R-PIA and carbachol thus validates the results obtained with TEA and 4-AP.

TEA antagonized in a concentration-dependent manner (0.1 to 10 mM) the negative effects of carbachol. The concentrations effective in our experiments are similar to those that prolong action potential in the guinea-pig ventricular fibres (Ochi & Nishiye, 1974), in guinea-pig ventricles (Corabeuf & Vassort, 1968) and in the heart of rat embryo (Bernard & Gargouïl, 1969). The mechanism of the negative effects of ACh in the mammalian heart (Ten Eick *et al.*, 1976) and in single atrial and pacemaker cells (Sakmann *et al.*, 1983; Iijima *et al.*, 1985) is dependent on the increase of the outward K^+ current shortening action potentials and indirectly reducing Ca^{2+} influx. The antagonism by TEA of carbachol in our experimental conditions is quite in accordance with the above data. However, TEA failed to inhibit the decrease of contraction and frequency induced by R-PIA. This result is puzzling, as the electrophysiological effects of Ado (and R-PIA) and of ACh in whole cell and patch-clamp studies are not distinguishable from each other and both substances activate the same K^+ channel population (Belardinelli & Isenberg, 1983; Kurachi *et al.*, 1986). Since our results with TEA are not in agreement with these conclusions, we studied the effect of 4-AP, by using the same protocol as for TEA. 4-AP inhibited the effects of carbachol, at concentrations which lengthened the action potential, increased the spike amplitude, and antagonized both the electrical and contractile effects of ACh in guinea-pig atria (Freeman, 1979). Again 4-AP was not able to antagonize the atrial effects of R-PIA.

A pertinent question is how can the difference between R-PIA and carbachol in atria be explained, if the two drugs are believed to affect the same population of K^+ channels? A first suggestion is that a block of K^+ channels unmasks the response of voltage-dependent Ca^{2+} channels (Hille, 1984). R-PIA is still effective in the presence of TEA or of 4-AP, implying a negative effect on Ca^{2+}-channels. This is further supported by the effect of TEA in experimental conditions where slow Ca^{2+} currents through L-channels are activated: 22 mM K^+ inhibits the fast Na^+ current thus abolishing the contractility of atria; the addition of noradrenaline together with suitable electrical stimulation induces slow action potentials chiefly due to slow Ca^{2+} currents (Pappano, 1970; Thyrum, 1974; Sada *et al.*, 1986). In such experimental conditions, carbachol is not effective, while R-PIA is still inhibitory.

R-PIA may antagonize the positive inotropism of Ca^{2+} addition by directly inhibiting the Ca^{2+} channels. These data are in accordance with previous results showing that R-PIA and other analogues of Ado are able to antagonize the positive inotropic effect of the dihydropyridine Ca^{2+} L-channel activator Bay K 8644 on isolated atria (Caparrotta *et al.*, 1987). Such effects are dependent on A_1 receptors and not on a direct interaction between Ado analogues and dihydropyridines at the level of specific binding sites on Ca^{2+} channels (Borea *et al.*, 1989). In addition, in rat cultured dorsal root ganglion neurones, 2-chloroadenosine reduces Ca^{2+} inward current activation by a direct effect on A_1-adenosine receptors (Dolphin *et al.*, 1986).

We suggest that Ado and ACh, although they have the same activity on ion currents in isolated cells or patch clamp studies, may display a different behaviour in atrial multicellular preparations under certain conditions, in that adenosine receptors may additionally inhibit Ca^{2+} channels.

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